

AD \_\_\_\_\_

Award Number: DAMD17-01-1-0568

TITLE: Pilot Study to Measure the Effects of NSAID Use on Angiogenesis and Apoptosis in Female Invasive Breast Cancer

PRINCIPAL INVESTIGATOR: John B. Richardson, Ph.D.  
M.C. Guiot  
V. Tagalakis  
E. Zorychta

CONTRACTING ORGANIZATION: McGill University  
Montreal, Quebec, Canada H3A 2T5

REPORT DATE: September 2003

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20040311 074

# REPORT DOCUMENTATION PAGE

*Form Approved  
OMB No. 074-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY (Leave blank)</b>			<b>2. REPORT DATE</b> September 2003		<b>3. REPORT TYPE AND DATES COVERED</b> Final (1 Sep 01-31 Aug 03)		
<b>4. TITLE AND SUBTITLE</b> Pilot Study to Measure the Effects of NSAID Use on Angiogenesis and Apoptosis in Female Invasive Breast Cancer			<b>5. FUNDING NUMBERS</b> DAMD17-01-1-0568				
<b>6. AUTHOR(S)</b> John B. Richardson, Ph.D. M.C. Guiot V. Tagalakis E. Zorychta							
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> McGill University Montreal, Quebec, Canada H3A 2T5  E-Mail: John.richardson@mcgill.ca			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>				
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>				
<b>11. SUPPLEMENTARY NOTES</b>							
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited					<b>12b. DISTRIBUTION CODE</b>		
<b>13. ABSTRACT (Maximum 200 Words)</b>  To examine the effects of NASIDs on invasive breast cancer we are performing a immunohistochemical analysis on 220 cases of breast cancer from Saskatchewan which has provided a complete drug history of each patient. We are assessing the tumours for COX 1 and COX 2, angiogenesis, estrogen receptor, proliferative index and the degree of apoptosis. Previous work has shown that some breast cancers over express cyclooxygenases COX 1 and 2 and a large epidemiological study conducted in Saskatchewan suggests that non-steroidal anti-inflammatory drugs may slow the growth of breast cancers and prevent metastases. The proposed mechanism for this effect is a decrease in angiogenesis or an increase in cell death. Our initial work on these cases has demonstrated obvious differences between the tumours. These morphological findings will be correlated with the clinical history of drug use when the complete analysis of all 220 cases is complete.							
<b>14. SUBJECT TERMS</b> breast cancer, immunohistchemistry, cyclooxygenase, nonsteroidal anti-inflammatory drugs					<b>15. NUMBER OF PAGES</b> 7		
					<b>16. PRICE CODE</b>		
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified		<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified		<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified		<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

## **Table of Contents**

<b>Cover.....</b>	<b>1</b>
<b>SF 298.....</b>	<b>2</b>
<b>Table of Contents.....</b>	<b>3</b>
<b>Introduction.....</b>	<b>4</b>
<b>Body.....</b>	<b>5</b>
<b>Key Research Accomplishments.....</b>	<b>5</b>
<b>Reportable Outcomes.....</b>	<b>6</b>
<b>Conclusions.....</b>	<b>6</b>
<b>References.....</b>	<b>6</b>
<b>Appendices.....</b>	

REPORT; GRANT No. DAMD17-01-1-0568INTRODUCTION:

Animal experiments and epidemiological studies, which were conducted in Saskatchewan Canada suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) slow the growth of breast cancers and prevent metastases. Some breast cancers over express cyclooxygenases COX 1 and COX 2 which, through prostaglandins, may have an effect on angiogenesis in the tumors. NSAIDs may inhibit the synthesis of the prostaglandins and thus modify the angiogenesis in a positive manner. Animal studies in mice indicated that these drugs do affect angiogenesis but also increased tumour cell death. The present study was proposed as a pilot study of stored pathological material to assess the expression of COX1 and COX 2, angiogenesis and apoptosis. This stored material was to be obtained from hospitals in the province of Saskatchewan in Canada where the complete drug history of the patients was documented in addition to their clinical record in regard to the breast carcinoma and subsequent outcome. This is a unique source of epidemiological material and previous studies on this problem have been published by one of the principal investigators in this pilot proposal, Dr. Colin Sharpe (1`)

The submission of this report was delayed for one year as a consequence of the death of one of the principal investigators, Dr. Colin Sharpe. Dr. Sharpe was responsible for the organization of the pathological material to be sent from Saskatchewan to Montreal for histological analysis. He had selected certain cases from a large study previously reported (1) which had shown a favorable outcome of patients who had taken nonsteroidal anti-inflammatory drugs during a period of several years before their carcinoma in both tumor size and the presence of distant metastases. Upon his death the transport of the histological blocks from the various hospitals in the province of Saskatchewan was placed on hold and no fixed tissue was available for the study as planned. Thus the original timetable was delayed.

In the fall of 2002 the Department of Epidemiology at McGill University found a replacement for Dr. Sharpe in Dr. V. Tagalakis, an Assistant Professor in that department and an investigator who was familiar with the work of Dr. Sharpe. Meetings with Dr. Tagalakis and Dr.J.P. Collet another colleague of Dr. Sharpe's during this period re-activated the program. The hospitals throughout Saskatchewan, which had already retrieved the paraffin embedded tissue blocks of the patients with metastatic carcinoma, agreed to forward the blocks to The Montreal Neurological Hospital where the histological and morphological work would be performed, This transfer began in December of 2002 and was complete by May 2003. At this point work on the analysis could begin. The project, as originally proposed, was thus delayed for a year and a half

due to this unfortunate event of the death of Dr. Sharpe.

5.

BODY:

In preparation for the work a variety of specific antibodies were tested in our histology laboratory to assess the proliferation index of the tumour with MIB-1, the expression of COX 1 and COX 2, the presence of apoptosis by expression of cleaved caspase 3, the degree of vascular proliferation as assessed by factor 8 or CD 31 and the presence of estrogen receptors. In addition a portion of the funds was used to purchase part of an automated immunostainer to perform the extensive immunohistochemical procedures. The purchase of this piece of equipment was financed mainly by the Montreal Neurological Institute whose investigators would also use the machine for research if required. By June of 2003 the most suitable antibodies had been selected and tested and control tissues obtained. The paraffin embedded blocks were then cut and routine H&E histological stained sections were made in order that the most suitable examples of the carcinomas could be selected for the immunohistochemical studies. The rationale for this initial review was the selection of slides which contained both the carcinoma and normal breast tissue which would act as a comparison and control to the carcinoma. The histological type of the carcinoma was also determined and if lymph nodes were present they were assessed for metastatic tumour. After this initial review of the material several slides were chosen from each cases for immunohistochemistry and these were then possessed with the above antibodies.

The investigators involved in this study from the Department of Pathology at McGill University who assist with the technical and interpretive aspects of the investigation are Drs. E. Zorychta and M.C. Guiot. Both are professors in that department. The epidemiological analysis is under the direction of Dr. V. Tagalakis also of McGill University.

KEY RESEARCH ACCOMPLISHMENTS:

An initial review of twenty five cases, out of a total of 220 cases, each case consisting of five to ten paraffin blocks, showed that there were clearly differences between normal and carcinoma and between different cases. These differences were graded by two independent pathologists and entered onto an Excel chart. A further twenty cases were then processed and again assessed and entered onto the chart. At this point in the study the coded cases have not been identified to the two pathologists and this final identification will not be performed until the entire 220 cases have been processed..

Several problems have arisen with the handling of this tissue which were not expected at the beginning. Many of the cases are old and the embedded material in mounting blocks which do not fit the microtomes used in our laboratory. This problem was solved by remounting the blocks after they had been melted down and then placed in new holders which were compatible with our microtomes. Another problem was the presence of needle biopsies which while not numerous did not contain normal breast tissue to act as a control for our immunostaining of the tumours. These cases were noted and this problem will be dealt with when the final analysis of the entire case load is finalized.

## REPORTABLE OUTCOMES:

6.

We are now processing the cases at a rate of ten to twenty cases per week depending on the availability of technical help and we plan to have the entire project completed by the end of 2003. The epidemiological correlation will be performed by Dr. Tagalakis and colleagues in the Department of Epidemiology at McGill and Dr. Tagalakis is ready to receive the data at the present time. The results to date indicate a very variable proliferation index of between 1% to 60% in the tumours. Apoptosis as assessed by caspase is not marked and angiogenesis was only increased in one carcinoma to date. COX 2 was elevated in about 50% of the cases and in only 10% was there expression of COX 2 in the normal tissue on the same slide. The most marked expression of COX 2 was found in a tumour with atypical hyperplasia associated with it.

The above initial findings with COX 2 are similar to those recently reported in patients with tumours and adjacent carcinoma in-situ (2) and are also in keeping with other studies in patients with breast carcinoma (3). When our study is completed correlation with the use of the nonsteroidal antiinflammatory drugs will be possible as neither of the two reported studies had such a correlation of drug use in their cases.

## CONCLUSIONS:

At the present time we are unable to come to definite conclusions as to the effects of NSAIDs on the outcome of breast carcinoma. The morphological findings to date indicate that there are definite differences in the cases of invasive breast cancer studied so far in the proliferation index and the expression of COX 2 in particular. These findings are encouraging as when the complete analysis is accomplished and correlated with the epidemiological data some definite conclusions are anticipated. The findings to date are similar to some recent reports but these reports lack the completeness of the study that we are engaged in since we have access to the entire drug history of the patients, their clinical courses and the eventual outcome plus the morphological material sent from the various hospitals across Saskatchewan. We anticipate publication of this material in early 2004 and this published material will be forwarded as a part of this initial report.

## REFERENCES:

1. Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, Hanley JA. Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage. Br J Cancer 2000;83:112-120.
2. Half E, Tang M, Gwyn K, Sahin A, Wathen K, Sinicrope FA. Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma in situ. Cancer Research 2002;62:1676-1681.

3. Ristimaki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, Joensuu H, Isola J. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Research* 2002;6:632-635.